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**Supplemental information**

**Self-assembly of protein superstructures by physical interactions  
under cytoplasm-like conditions**

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# Supplementary Information for “Self-assembly of protein superstructures by physical interactions under cytoplasm-like conditions”

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## Supplementary Figures

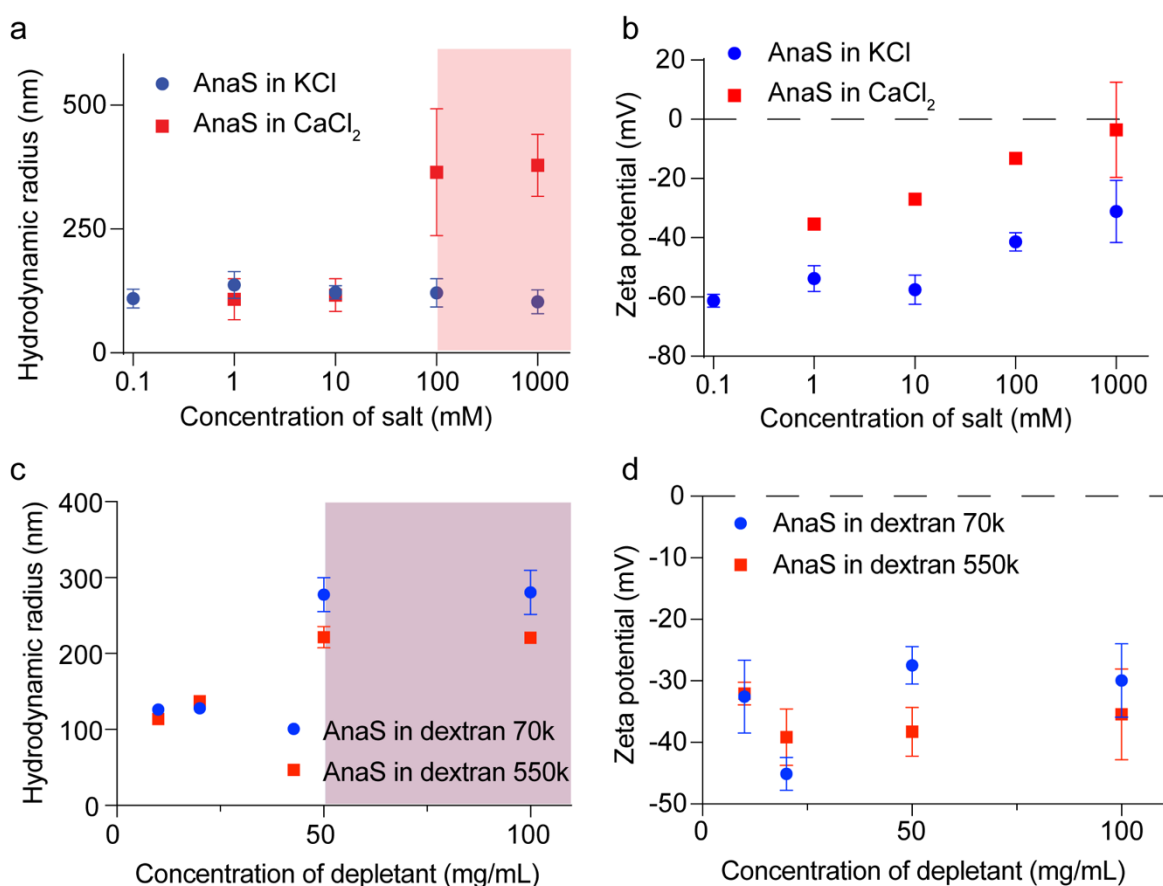


FIGURE S1. Self-assembly of AnaS with volume fraction at 0.04 vol/vol% in different concentrations of the electrolyte or depletant. The hydrodynamic radius (a,c) and zeta potential (b,d) of AnaS assembly in different electrolyte (a,b) or depletant (c,d) concentrations measured by dynamic light scattering (DLS). The shaded area in (a) and (c) denotes the concentration ranges for different electrolytes that can assemble GVs. All measurements are based on an average of 3 biological replicates. Error bars represent  $\pm$ SEM, where not seen, are hidden by symbols.

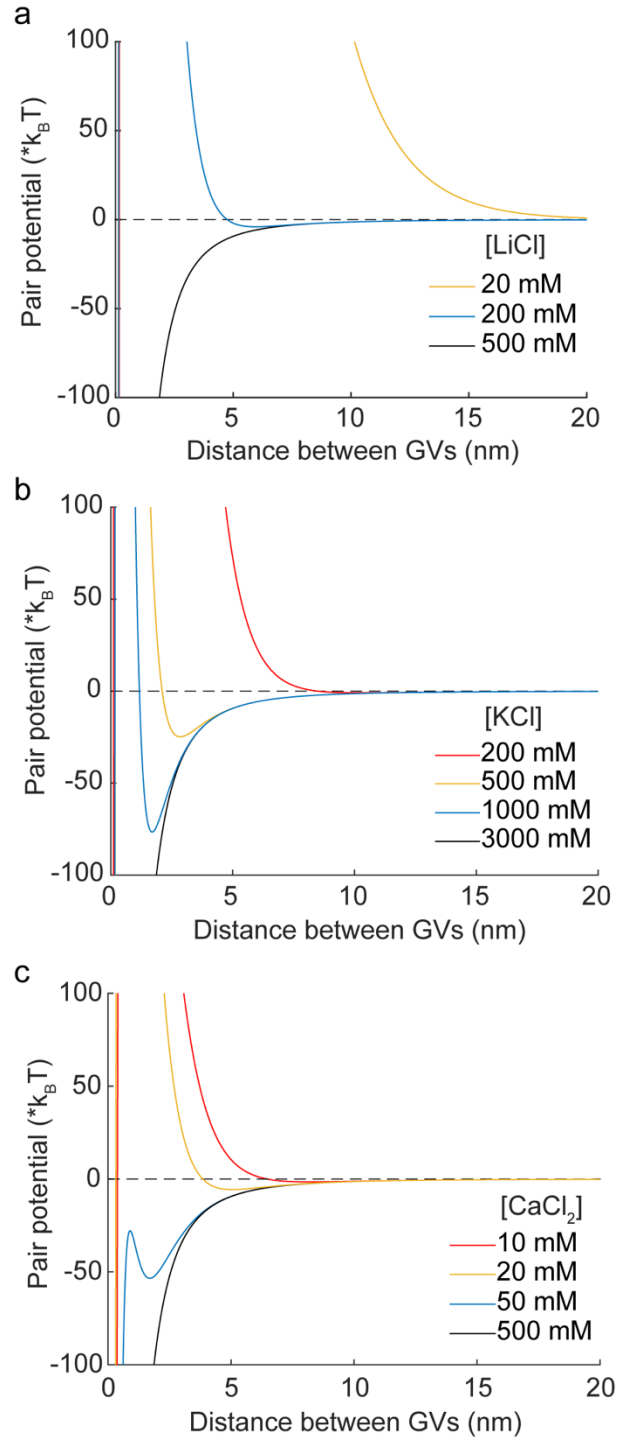


FIGURE S2. The free energy of a pair of AnaWT GV's in different concentrations of (a) LiCl, (b) KCl, and (c)  $\text{CaCl}_2$  considering only Derjaguin-Landau-Verwey-Overbeek (DLVO) theory.

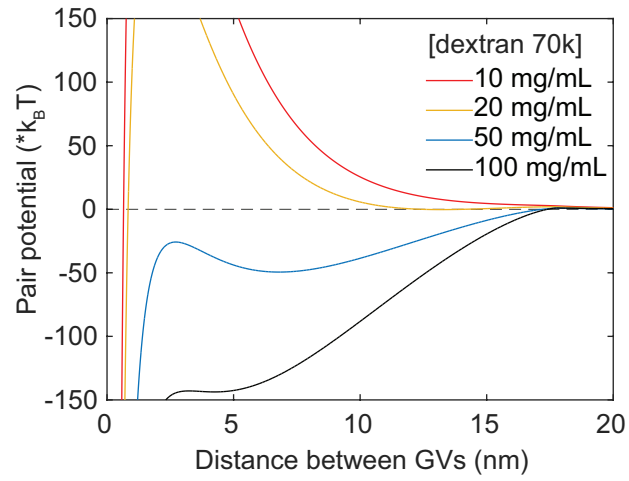


FIGURE S3. The free energy of a pair of AnaWT GVs in solutions with different dextran 70k concentrations, considering both DLVO and Asakura-Oosawa theory.

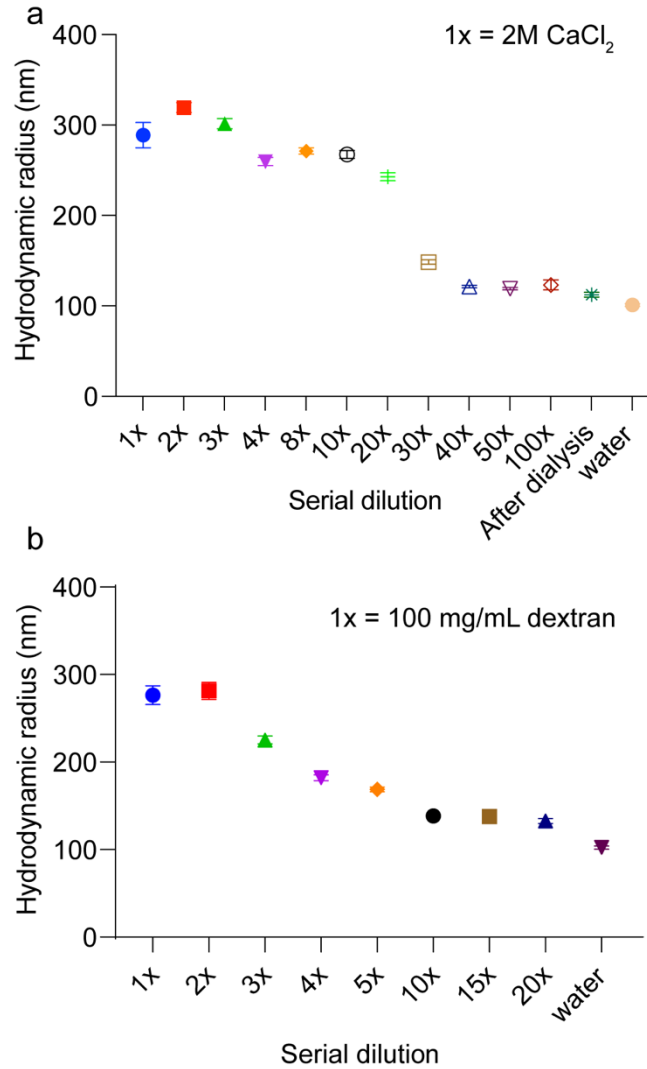


FIGURE S4. Disassembly of AnaWT GVs with 0.04 vol/vol% by diluting the solution from 2 M  $\text{CaCl}_2$  (a) or 100 mg/mL dextran 550k (b). GVs can be redispersed once the concentration of the  $\text{CaCl}_2$  or dextran drops below the threshold concentration shown in Fig. 2 and 3. This result demonstrates that GV assembly processes are reversible and the conditions in which GVs will assemble can be readily predicted by our simple thermodynamic models. All measurements are based on an average of 3 biological replicates. Error bars represent  $\pm\text{SEM}$ , where not seen, are hidden by symbols.

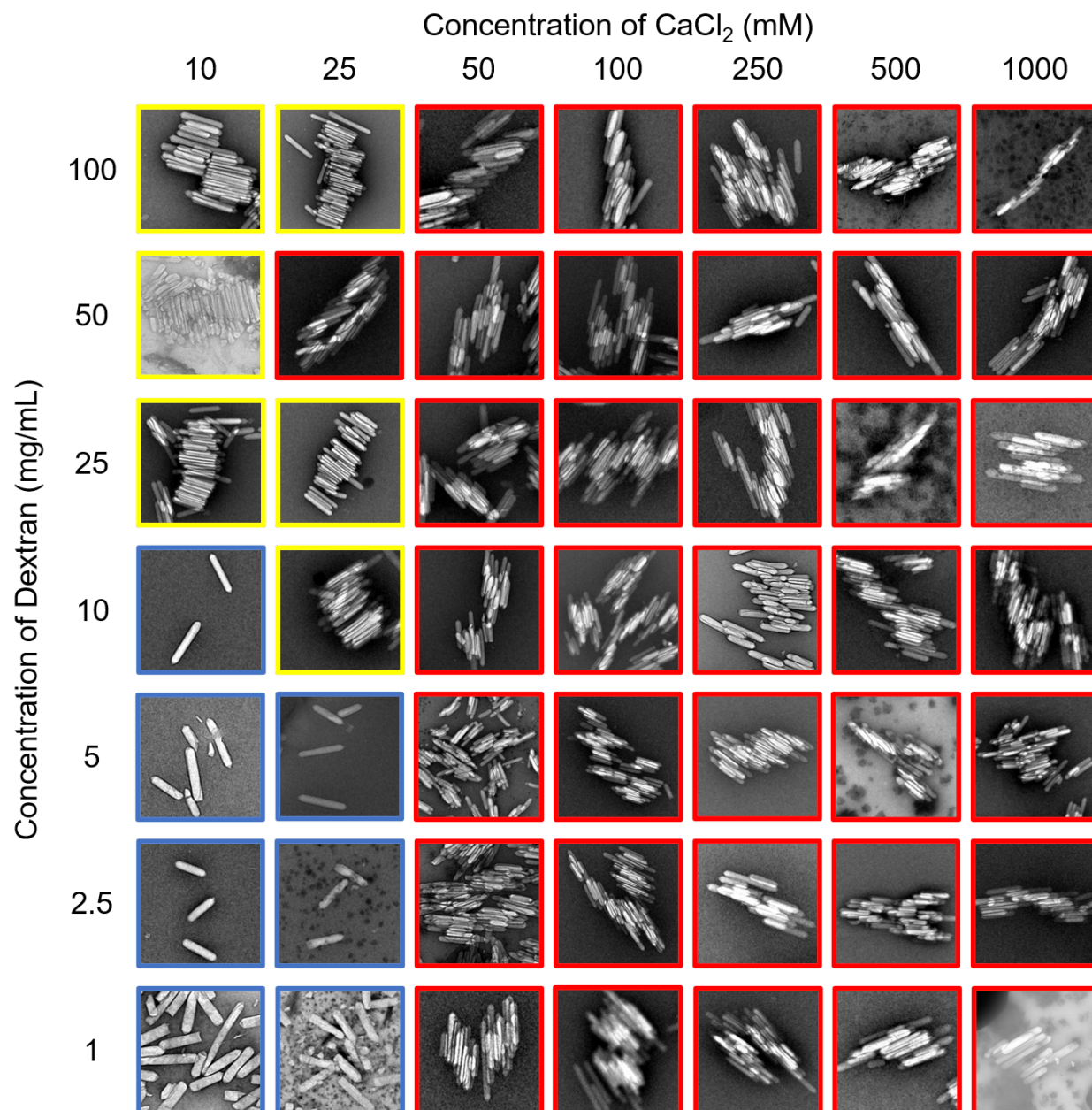


FIGURE S5. Representative TEM images of AnaWT assemblies in different concentrations of  $\text{CaCl}_2$  and dextran 550k in aqueous solutions at volume fraction 0.04%.

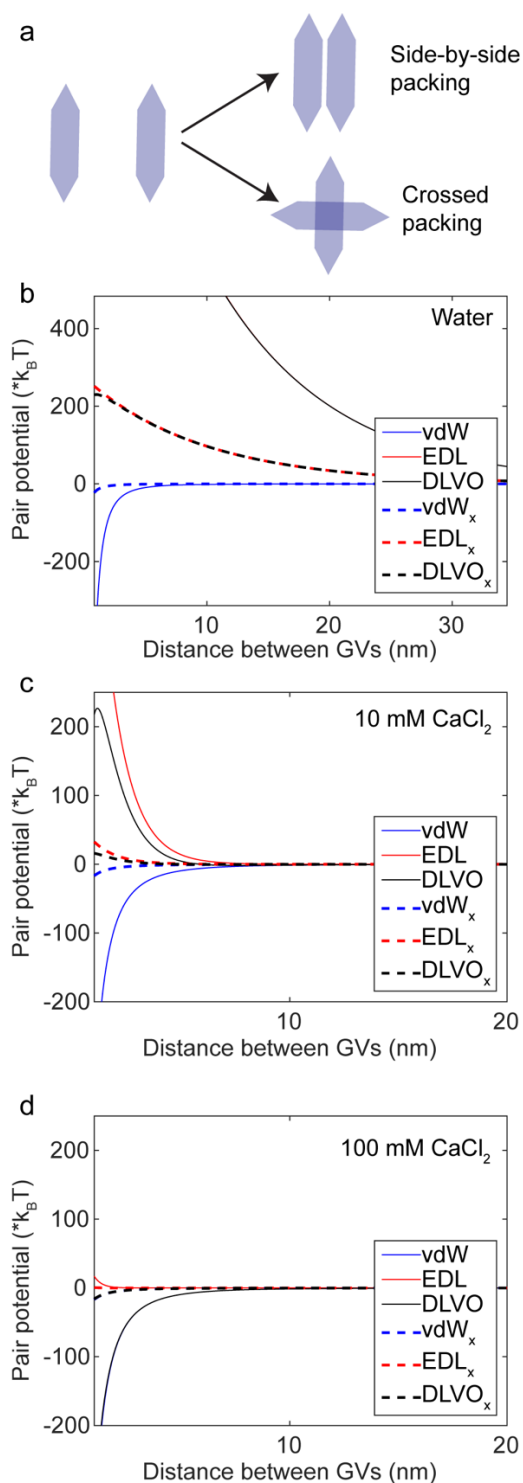


FIGURE S6. (a) Diagrams of GV assemblies with side-by-side and crossed packing configurations. Free energy calculation of a pair of GVs with parallel (solid lines) or crossed (dashed lines, marked with a subscript 'x' in the legend) packing configurations in water (b), 10 mM  $\text{CaCl}_2$  (c), and 100 mM  $\text{CaCl}_2$  (d), considering only DLVO theory.

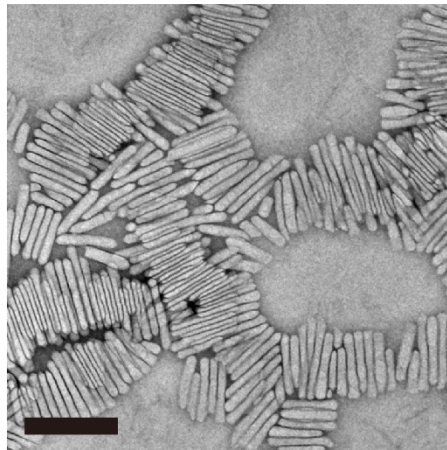


FIGURE S7 Representative TEM image of AnaWT GVs at volume fraction 0.04 % in 200 mM KCl and 50 mg/mL of dextran 550k. Scale bars, 1  $\mu\text{m}$ .



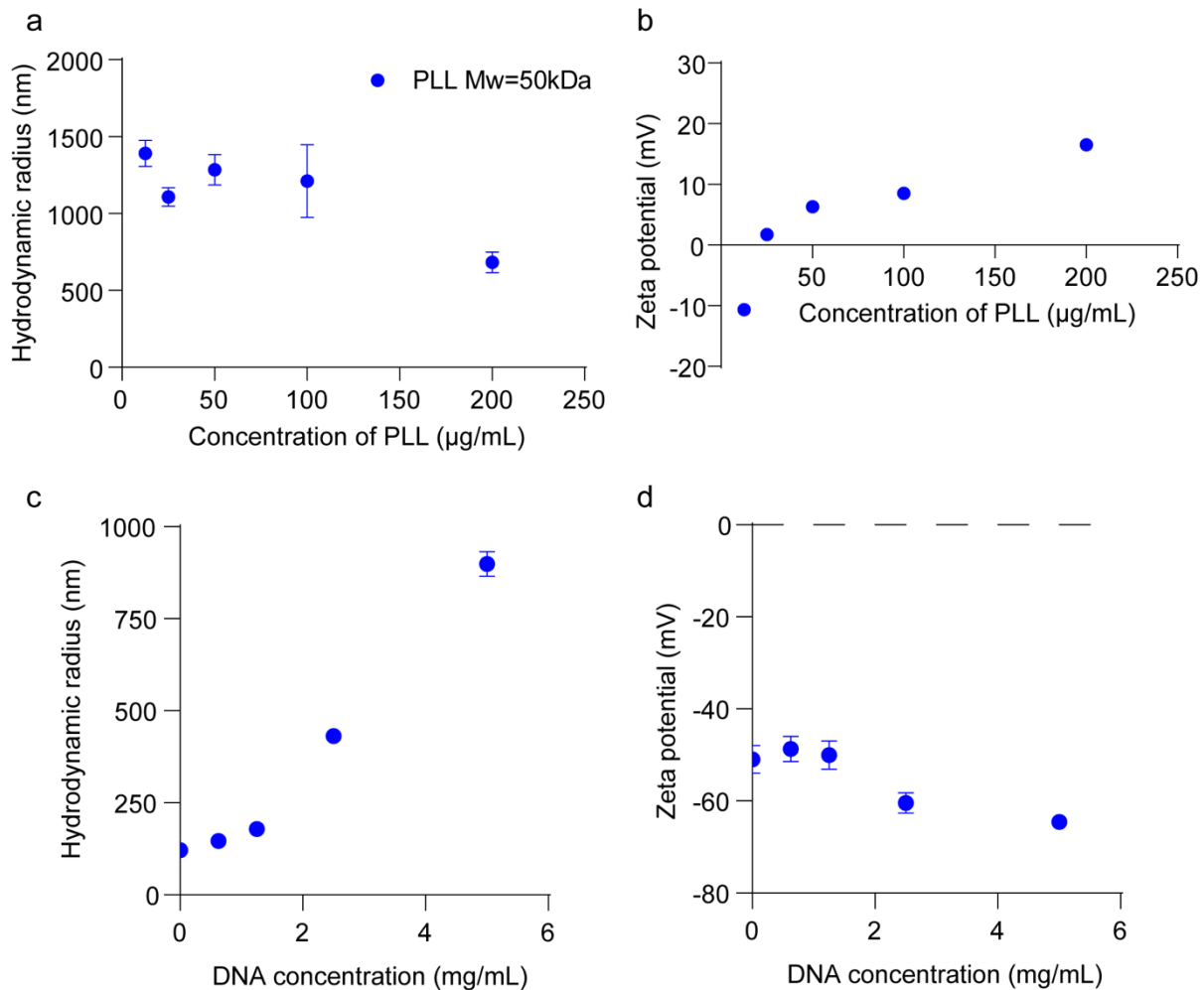


FIGURE S8. DLS (a) and zeta-potential (b) of AnaWT GV in different concentrations of polylysine (PLL) aqueous solution. DLS (c) and zeta-potential (d) of AnaWT in different concentrations of Herring Sperm DNA solution. The volume fraction of GV is at 0.04% for all experiments. All measurements are based on an average of 3 biological replicates. Error bars represent  $\pm$ SEM, where not seen, are hidden by symbols.